

EVALUATION EFFICIENCY OF GARLIC AND COLOCYNTH AS CRUED EXTRACTION AND SOME ANTIFUNGAL EFFECTS ON FUNGUS *CANDIDA ALBICANS*, WHICH ISOLATED FROM PATIENTS OF DIYALA PROVINCE TOWNS .IRAQ

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ABSTRACT

The results demonstrate a high inhibitory activity in the crude extracts taken from garlic (*Allium sativum*) and the colocynth (*Citrullus colocynthus*) against the fungus *Candida albicans*. These results also display of the minimal fungicidal concentration in the antifungal Fluconazole and Nystatin 50µg/ml. The colocynth extracts display a cell toxicity. This has been attained through the analysis of blood cells whereas the garlic extracts do not show cell toxicity in the blood cells. However, the hexanes extracts from colocynth and garlic show high percentages amounting to 52% and 63% respectively. The alcoholic and hexanes extracts for these two plants gave the best inhibitory effectiveness by comparison with cold and hot water extracts. Moreover, the garlic extracts show a better inhibitory effectiveness than that shown by the colocynth extracts. The hexanes extracts for garlic extracts gave highest inhibitory percentage amounting to 98.8% at the concentration of 100 µg/ml, whereas a lowest inhibitory percentage are shown by the cold water extracts for the colocynth, i-e 57.6 % at the concentration of 20 µg /ml. The concentration 100 µg / ml for the hexanes extracts of the garlic gave an inhibitory effectiveness equal to that shown by antifungal Nystatin at the concentration 2 mg /ml.

KEYWORDS: Plant Extracts, Garlic, Colocynth, Antifungal and *Candida albicans* Fungus

INTRODUCTION

The yeast *Candida albicans* exist naturally in the mucous membrane of the mouth, the uterus and the alimentary canal, which turn into an infection organism within certain circumstances. Thus it infects a human being with certain maladies like the AIDS, diabetes, leukemia, etc. These, however, will require the use of antibiotics on a large scale, or the use of chemotherapy by those affected with cancer, and during pregnancy (Santos et al 2001; Brook et al 2001). The yeast *C.albicans* is characterized by being dimorphic and its grows in its yeast oval form or in its mycelia from depending on favorable environmental circumstances including heat and the hydrogen number (pH), humidity as well as the components of the nutrition's medium. The yeast form grows on the solid acid vegetation containing organic nitrogen and sugary substances as a source of carbon in degrees less than 35 c. The mycelia form grows in cultures containing non-organic compounds such as Zapeks Dox Ager in addition to the cultures comprising starchy substances like Corn Meal Ager- Moreover, the Potato Dextrose Ager which has a hydrogen number amounting to 6.5or even more (Odd, 1979). However, the capability of pathogenicity possessed by *C.albicans* is ascribed to its adherence. It also forms germ tube as well as produces protein-digestive enzyme. It also contribute to the phospholipase and the phenotypic switching (Abo- Elteen 2001; Kevin and Gary 2000). Pharamacognosy deals with the medical features of plants in order to get natural antibiotics like Aspirin extracted from willow as well as the antibiotic Opioids which is extracted from *Opium poppies* and steroid structures extracted from sweet potato in Mexico and others (Mills et al 2006). In this study garlic (*Allium sativum*) and colocynth (*Citrullus colocynthus*) were used for testing. The study aims at the comparison between

cruded plant extracts taken from *A.sativum* and *C.colocynthus* which are characterized by being inhibitory to the growth of fungus *C.albicans*, which, in turn, are compared with some antifungal.

MATERIALS AND METHODS

The experiment was conducted in the laboratories of the Higher Studies, the department of biology, college of Education/Al- Razi, the University of Diyala. As for the samples used, the researches has managed to obtain 97 samples taken from different parts of human body like the mouth, medial ear, skin, nails, uterus, urine, etc. All the samples have been collected randomly from patients in some cities and towns in the Province of Diyala including Baqubah (Baqubah Teaching General Hospital as well as Al-Batol Obstetrics and Pediatrics Hospital), the town of Kan'an (the Health Center) in the period extending from July 2010 until February 2011. The samples were placed in sterile test tubes containing salty liquid NaCl at a concentration of 0.1M. The samples were preserved in refrigerators until they were transferred to the laboratory for checkup and diagnosis. The samples, however, were planted in the Sabouraud Dextrose Ager (SDA) which was prepared by Emmons and others (1977). These samples were sterilized in plastic plates which were incubated at 25- 30 Celsius for 2 to 4 days .The fungus was diagnosed according to Cletus and Jack (1998). This, however was emphasized by using vegetation and chemo biology for the fungus *C.albicans*, especially in its potentiality to consume the Carbon source with the existence or absence of Oxygen (Cletus & Jack, 1998; Milne, 1996).

Testing the Sensitivity of *C.albicans* to Antifungal

The effectiveness of antifungal Nystatin, Ketaconazole and Fluconazole was tested to detect the minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC) by using the liquid sabouraud by Broth dilution method. The antifungal were used in their pure powdery from prepared by dissolving 0.0010 grams of the antifungal in 10 ml of an organic solvent Dimethyl Sulphoxide From which other solvents were derived. A series of circumstances for the antifungal 0.05- 50µg/ml, were prepared .This was done after the main concentration of the antifungal at 100 µg/ml. However, 12 test- tubes containing 2 ml each were taken out of Sabouraud Dextrose Broth (SDB), then 2ml of main antifungal was added to the first test to obtain the concentration 50 µg/ml .This concentration was used to derive other concentrations required by multiple dilution while the positive control tube, and the negative control tube. Every test tube was pollinated, then these test tubes were incubated at 30 C for 48 hours. The test tubes were shaken into a mix after that. The growth is seen through the comparison of test tubes with the control tube. The values of MIC in which no growth was observed were registered. The value of MFC was assessed through the planting of 0.1 ml from every tube in which no contain growth and control tube on media with no antifungal .After incubation at 30 c for 48 hours. The test done after the growth of fungi in control tube observed. MFC represent the lowest concentration for antifungal which give a negative result after second implantation as shown by Shadomy et al (1985).

Methods for Plants Extraction

For different kinds of crud plant extraction prepared from fruity parts of garlic and colocynth plants, which are Alcoholic, Hexane, Cold water and Hot water extracts according to the following extraction methods:

Alcoholic Extract

Shtayeh and Abu- Ghadeib (1999) method was followed by weighting 10 g of plant powder and was put into a flask valued 250 ml and added to it Ethanol 70% , The mixture was put in a shaking incubator for 24 hours with temperature 35 c. After that the mixture was filtered using Gauze Sponges. The flitter was distributed in centrifuge tubes with speed 3000 r/m for 10 minutes. The filtered liquid was gathered and put into big surface plates; the alcohol was dried

in an oven with temperature 40 c until the alcohol evaporates completely to obtain a powder from alcoholic extract. The mixture was put into dark tubes made from glass and firmly closed. It was kept in freezer with temperature – 20 c to ready when needed to use. The method was repeated several times to get enough quantity of plant extract. As for microbic tests the concentrates 0.0, 20, 40, 60, 80, 100 mg/ml and was sterilized by using Millipore filter with holes 0.22 micrometer.

Hexanes Extraction

The same above method was used by using hexane extraction instead of alcoholic extraction.

Cold Water Extraction

Parekh and Chanda (2007) method was used by taking 10 g of plant rowdier and was put into a flask 250 ml, added to it 100 ml of sterilize water. The mixture was shacked in a shaking incubator for 24 hours and temperature 37 c. The mixture was filtered using Gauze Sponges and was put into tube centrifuged with speed 5000 r/m for 10 minutes and the liquid was filtered by filtering papers. The mixture was gathered in glass plates diameter 20 cm. The mixture was entered into an oven with 40 c to avoid extra water and to obtain dry powder extraction. It was kept into dark glass bottles and firmly closed and entered into a freezer with – 20 c to ready in use when needed. The operation was repeated several times to gain enough quantity of cold

Hot Water Extraction

El- Fallal and El- Kattan (1977) method was followed by taking 10 g of plant powder and put into a flask 250 ml, added to it 100 ml of sterilized boiled water. The mixture was shacked in a shaking incubator for 30 minutes with temperature 28 c. The mixture was filtered by using Gauze Sponges and put it tubes that were centrifuged in speed 3000 r/m for 10 minutes, then the placid was gathered in flasks with diameter 25 cm and was entered in an oven on temperature 40 c to avoid extra water in order to obtain dry powder. It was kept in dark glass bottles in freezer with temperature – 20 c to ready when needed.

Estimating Cellules Toxicity for Plant Extracts

The Xin-guo and Ursella (1994) method was followed by putting 0.8 ml from each extract in a sterilized test tube and added to it 0.2 ml of human red blood cells so that the final value will be 1 ml. The tube was shacked well and incubated in an incubation for 30 minutes with 37 c temperature, after that they were centrifuge by using centrifuge for 5 minutes with 1000 r/m. Hemolysis has been noticed in contrast with control treatment (test tube with blood only) to observe the difference in hemolysis.

Estimating Percentage and Acid Function for Both Garlic and Colocynth Plant Extracts

The Percentage rates for the prepared plant extracts were estimated according to Al- Balane (2003) method as follow:

$$\text{Extract percentage} = \frac{\text{Weight of extract}}{\text{Weight of extract}} \times 100$$

And the pH determination was measured for the plant extract after operation of drying and solving.

Test Inhibition Effectiveness of Plant Extract in *C.albicans* Growth

It has been soured that the plant extract has not been contaminated by planting 0.01 ml of plant extract in Sabouraud Dextrose Ager (SDA). The SDA was incubated in an incubation for 3-7 days with 37 c, El- Kady et al (1993)

method was followed in doing this. The dry extracts were mixed with cold and solved SDA to temperature 40 c with concentrates 20, 40, 60, 80, 100 mg/ml with two repeating in each concentrate. After the SDA was hardened a disc with diameter 5 mm. was taken from fungi colony and taken from growing fungi colonies on SDA for 7 days were the fungi disc was situated in the center of the disc.

Two types of comparing were used positive were init Nystatin was added with concentrate 2 mg/ml to a plate containing hard Sabouraud (SDA) and a negative concentrate with concluded (SDA) only.

The plates were planted in fungi and incubated with temperature 28- 30 c for a week. They diameter of growth colony was measured (two crossed diameter), and the results were written down and rate of frustration was measured by using the following equation:

$$\text{Inhibition percentage} = \frac{\text{diameter of fungi in comparing plates} - \text{diameter of fungi in treatment pla}}{\text{Diameter of fungi in comparing plates}}$$

Statistical Analysis

The data were analyzed by a practical experiment using CRD and the significant difference LSD with level 0.01 and the SPSS program was used to analyze data

RESULTS AND DISCUSSIONS

Testing Sensitivity of *C.albicans* to Antifungal

The results in table 1 showed that Fluconazole has best inhibition effectiveness were its lower inhibition concentration was 12.5 µg/ml, following it Keyaconazole and Nystatin with lower inhibition effectiveness 25 µg/ml against *C.albicans*. These results agree with that Al- Saidy et al (2012) and Danboos (2011) has reached to, and the results disagree with Al- Sadig (2006) has reached to, by the lower inhibition effectiveness concentrate for Nystatin 32 µg/ml against *C.albicans*.

This is also to the wide use and random use of antifungal which lead to the appearance of differences in sensitivity between different kinds of the same species, These differences lead to the appearance of different structure of breeds from wiled breeds (Devkatte et al, 2005; Godoy et al, 2003). The lower fungicidal concentrate for antifungal was 50 µg/ml.

Table 1: Values MIC and MIC Antifungal against *C.albicans*

Fungi	Antifungal	Values of MICµg/ml	Values of MFC µg/ml
<i>C.albicans</i>	Fluconazole	12.5	50
	Ketaconazole	25	50
	Nystatin	25	50

Estimating Cellules Toxicity for Alcoholic, Hexane, and Watery Extracts

The results in table 2 showed that plants extracts Of alcoholic, Hexane, and Watery garlic has effective toxic cellular of human red blood cells by Hemolysis with appeared in (Invetro) tests. Acts of Alcoholic, Hexane, and Watery garlic showed no toxic cellular of red blood cells.

This is due to lower concentrates of soapwort compounds in garlic. The appearances of toxic cellular is due to effectiveness of soapwort with sytrolite with constitutes the plasma cell from which causes the remove of plasma frame and frees Hemoglobin (Mills et al, 2006).

Extract Method	Toxic Function for Garlic	Toxic Function for Colocynth
Cold Water Extract	-	+
Hot Water Extract	-	+
Alcoholic Extract	-	+
Hexanes Extract	-	+

- = No existence of blood analysis (no toxic)

Extract Method	Percentage		Acid Function	
	Colocynth	Garlic	Colocynth	Garlic
Alcoholic extract	50	45	5.3	5.63
Hexanes extract	52	63	4.66	5.47
Hot water extract	33	35	5.45	5.54
Cold water extract	25	30	5.66	4.56

Concentrate Mg/ml	Diameter of Colonies Growth (mm)							LSD
Extract Method	0.0	20	40	60	80	100	Con.	Extract Method
Cold Water Extract	46.0	19.5	12.5	8.5	7.0	3.5	0.0	And
Hot Water Extract	45.5	18.5	17.5	10.0	7.0	2.5	0.0	Concentration =2.31
Alcoholic Extract	44.0	12.5	10.0	6.0	4.0	1.5	0.0	
Hexanes Extract	45.5	13.5	11.5	7.0	5.5	2.0	0.0	
Method =0.8	Concentrate = 1.18							P < 0.01

The results in table (5) showed significant differences between methods of extraction and the alcoholic extracts for colocynth plant and higher inhibition effectiveness than other extracts and the inhibition effectiveness of colocynth plant extracts with concentrates 100 mg/ml are equaled inhibition effectiveness of antifungal Nystatin 2 mg/ml. The highest inhibition rate was 96.56% with concentrate 100 mg/ml in alcoholic extracts, as for the lowest inhibition rate 57.60% was cold water extracts with concentration 20 mg/ml.

Table 5: Inhibition Effectiveness for Alcoholic, Hexane ,Cold Water and Hot Water Extract for Colocynth Plant against *C.albicans*

Concentrate Mg/ml	Diameter of Colonies Growth (mm)							LSD
Extract Method	0.0	20	40	60	80	100	Con.	Extract Method
Cold Water Extract	46.0	18.0	12.5	8.5	6.5	2.5	0.0	And
Hot Water Extract	40.0	14.0	8.5	8.0	6.0	2.0	0.0	Concentration =2.31
Alcoholic Extract	41.5	13.0	9.5	8.0	5.0	1.0	0.0	
Hexanes Extract	42.0	13.0	8.5	7.5	4.0	0.5	0.0	
Method =0.8	Concentrate = 1.18							P < 0.01

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